

Developmental effects of perinatal exposure to extremely weak 7 Hz magnetic fields and nitric oxide modulation in the Wistar albino rat

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Received 31 May 2007; received in revised form 2 September 2007; accepted 4 September 2007

Abstract

Prenatal exposure of pregnant dams to oscillating magnetic fields can cause behavioural deficits in their offspring which persist into adulthood. These changes are waveform-specific and may involve nitric oxide. To investigate the interaction between nitric oxide modulation and perinatal magnetic fields, dams were exposed from 2 days before to 14 days after birth to one of six magnetic field conditions (1, 5, 10, 50 or 500 nT or sham) and given either water, 1 g/L nitric oxide precursor L-arginine or 0.5 g/L nitric oxide synthase inhibitor *n*-methylarginine. At weaning (22d), their offspring were placed in the open field for observation. Rats given 50 nT field or 500 nT field + water were hyperactive and showed increased rearing and bodyweight. These strong effects were attenuated or absent in groups given 50 or 500 nT field + *n*-methylarginine. Groups given sham field + L-arginine were behaviourally similar to animals given 50 or 500 nT field + water. Higher intensity fields showed robust behavioural and physiological effects. In general, these effects were counteracted by co-administration of nitric oxide synthase inhibitor *n*-methylarginine, which had little effect on its own. Shams given NO precursor L-arginine were highly similar to those given any higher intensity magnetic field. Results support a critical developmental role of NO and the involvement of NO in magnetic field effects.

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Keywords: Magnetic fields; Nitric oxide (NO); L-Arginine; *n*-Methylarginine; Open field behaviour; Perinatal

1. Introduction

Nitric oxide (NO), a free labile gas (Snyder and Dawson, 1995), has a multitude of essential peripheral and central functions (Schuman and Madison, 1994) and plays a critical role in nervous system development (Gibbs, 2003). NO in tissue is synthesized from the amino acid L-arginine (LA) and citrulline by one of three isoforms of the enzyme Nitric Oxide Synthase or NOS (Stuehr, 1999). NO production can be reduced by synthetic NO inhibitors of these isoforms (Alderton et al., 2001) or increased by NO precursors or donors (Scatena et al.,

2005). Other biologically relevant stimuli can upregulate NO production, including extremely low frequency electromagnetic fields (EMF < 100 Hz) applied singly (Jeong et al., 2006; Jelenkovic et al., 2006; Kim et al., 2005) or in combination with lipopolysaccharides (Yoshikawa et al., 2000). These collective findings, which have been produced by multiple research teams working separately, have shown EMF-induced NO activation in many species, including rats (Jelenkovic et al., 2006) and mice (Jeong et al., 2006). Static magnetic fields may also have the ability to influence NO levels in humans (Sirmatel et al., 2006).

A wide spectrum of effects of EMF exposure has been identified over the past four decades (Persinger et al., 1973; Adey, 1981; Sienkiewicz et al., 2005) but only recently has NO been shown to play a role in the generation of these responses. Only a brief review of these many experiments is presented here; an extensive discussion exists elsewhere (Whissell and Persinger, accepted). Bawin and colleagues were the first to convincingly demonstrate the role of NO in tissue response to EMF (Bawin et al., 1996) when they showed that pharmacological inhibition

Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; EMF, extremely low frequency electromagnetic field; LA, L-arginine; NMA, *n*-methylarginine; Sh, Sham.

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of NO abolished the ability of 1 Hz magnetic fields to destabilize hippocampal slow rhythms. Subsequently NO was shown to play a role in the effects of EMF on nociception (Kavaliers et al., 1998; Dixon and Persinger, 2001; Jeong et al., 2006) and in the behavioural anomalies resulting from prenatal EMF exposure (McKay et al., 2003a). In addition to involvement of NO, EMF may require intact functioning of Ca^{2+} channels (Morgando-Valle et al., 1998; Jeong et al., 2006). EMF may also interact with the effects of NO modulation, as suggested by the ability of static magnetic fields to attenuate the vascular effects of NO inhibition (Okano and Ohkubo, 2003).

Striking long-term behavioural deficits are exhibited by pups of rat dams exposed during gestation to weak (<3 mT) EMF during pregnancy (Persinger, 1969; Ossenkopp, 1972; Persinger et al., 2001; McKay et al., 2003a,b). These alterations were specific to the applied waveform, but changes in activity level in the open field were frequently reported. Comparable effects on activity result from exposure of rats to NOS inhibitor *N*-nitro-L-arginine methyl ester (L-NAME) during the perinatal period (Prickaerts et al., 1998). Recently, McKay and others found that NOS activity played a central role in EMF-induced developmental anomalies (McKay et al., 2003a). They observed that the offspring of dams treated with a 0.5 Hz, microT-range EMF were hyperactive as pups but those co-administered the EMF and L-NAME postnatal 14d were not. To fully illustrate the role of NO in EMF effects, these findings will have to be generalized to other NO inhibitors and waveforms.

To date no studies have examined the physiological interactions between LA availability and EMF in the perinatal context. Given the role of LA in NO production (Stuehr, 1999), the effects of LA on the perinatal environment (Racasan et al., 2004; Xiao and Li, 2005) and the growing popularity of LA supplementation (Davidson and Geohas, 2003), the role of LA in EMF response warrants investigation. Similarly, little is known about interactions between EMF and other NOS inhibitors such as *n*-methylarginine or NMA (Gold et al., 1990). While acute NO modulation has few behavioural consequences in adulthood (Wortwein et al., 1997), chronic NO inhibition is of concern as it incurs long-term growth retardation (Voelker et al., 1995; McKay et al., 2003a) and is relevant to EMF.

The current study examines the interaction between exposure of dams to a 7 Hz, square-wave EMF and NO modulation with NO inhibitor NMA (0.5 g/L) or NO precursor LA (1.0 g/L) from 2 days before to 14 days after birth. The 7 Hz frequency within a narrow intensity band around 50 nT has previously been shown to be therapeutic in models of multiple sclerosis (Cook and Persinger, 2000; Kinoshameg and Persinger, 2004), may be linked to seizure activity and sudden infant death (Persinger et al., 2005) and acts as a zeitgeber for many biologically important functions (Cherry, 2002). Field intensities of 5, 50 and 500 nT were used in order to address the possibility of intensity windowing (Blackman, 2006). The open field test, a measure of general rat behaviour, was used to detect any developmental anomalies. This measure is jointly sensitive to NO modulation (Prickaerts et al., 1998) and EMF exposure during the perinatal period (Persinger, 1969; Ossenkopp, 1972; Ossenkopp and Ossenkopp, 1983; Persinger et al., 2001; McKay et al., 2003a). If the EMF used is

effective and depends on NO activation, it should be reversed by administration of NMA and interact with LA supplementation.

2. Experimental procedures

2.1. Subjects

In five separate blocks of experiments over a 2-year period a total of 36 albino Wistar female rats (12/block) obtained from Charles River Breeders (Quebec) were exposed singly in 46 cm $\times$ 26 cm $\times$ 19 cm plastic cages with metal tops. In each block, half of the females were maintained in one of two aluminum holding racks. Each rack has been described previously (Stewart and Persinger, 1999). In summary, the perimeter of each rack was 112 cm $\times$ 125 cm and wrapped with 72 turns of 30 gauge American wire resulting in a width of 13 cm. To insure pregnancy, each female was housed with a 4–5-month-old male for 10 days after which time he was removed. Females were between 4 and 5 months old at the time of parturition. The room temperature was maintained at 21 ± 1 °C and the light:dark cycle was 12:12 with onset at 08:30 LT. Food was available *ad libitum*.

2.2. Pharmacological NO modulation

From 2 to 3 days before birth to 14 days after birth the mothers were given access to either tap water, 1 g/L of L-arginine (LA, Sigma) in the tap water, or 0.5 g/L *n*-methylarginine (NMA, Sigma) in tap water. These fluids were measured and refreshed daily. Daily measurement showed no conspicuous differences between fluid consumption between these three treatments.

2.3. Magnetic field exposure and testing

During the period the fluids were administered one of the two coiled racks was activated by a 7 Hz square wave from a Heath Schlumberger function generator whose output was delivered through an attenuator. Maximum exposure intensity was maintained at either 5, 50 or 500 nT. Even though the two racks were orthogonal within the room (east-west vs. north-south orientation) and separated by 2 m, an induction occurred within the coil of the non-activated rack. According to a MEDA FM 300 Vector magnetometer, the maximum “induced” fields within this coil from activated coils with 5, 50, and 500 nT fields were <1 nT (designated the sham group), 1, and 10 nT, respectively.

During all experiments the sensor for the magnetometer was maintained within a plastic cage with a wire top, identical to the mothers’ exposure cages, while local magnetic field measurements for the *X* (direction of the applied field: 13,800 to 14,200 nT), *Y* (perpendicular to the applied field: 18,850 to 21,000 nT), and *Z* (vertical: 39,200 to 41,800 nT) components and the declination and inclination were sampled and stored every 24 s on a laptop computer. The presence of the experimental field was verified by the continuous magnetometric record as well as several times during the day and night by placing a magnetic coil for telephone recording coupled to an acoustic amplifier into the coil containing the cages in order to hear the magnetic signature of the 7 Hz pulse. Direct measurements indicated that the presence of the stainless steel wire top of the cage did not attenuate the intensity of the fields in either coil.

Following birth the numbers of pups per litter were monitored daily with minimal disturbance. At 14 days of age the magnetic fields were discontinued and all mothers and litters were given tap water. At 22 days of age each rat was tested singly for open field behaviour. Testing began by randomly selecting a litter from those currently 22 days of age and then testing all rats within that litter. Any other litters of this age, if present, were then selected and tested to completion one by one. All testing was done between the hours of 13:00 h and 17:00 h by a single experimenter blind to condition.

3. Results

Preliminary analysis was performed utilizing a 2 (sex: male or female) $\times$ 3 (drug: water, LA or NMA) $\times$ 6 (field intensity: 0, 1, 5, 10, 50 or 500 nT) Multiple Analysis of Variance (MANOVA) for movement, rearing, grooming, defecation,

urination and bodyweight and was shown to be significant ($p < .05$). A $2 \times 3 \times 6$ ANOVA was then performed for each variable and is presented below. This was followed by a Least Significant Differences test (LSD, $p < .05$) to examine any main effects or interactions *post-hoc*. All analyses were performed using SPSS software on a VAX 4000 computer.

3.1. Movement

Three-way ANOVA for movement detected main effects for sex [$F(1,403) = 4.90$, $p < .05$], drug [$F(2,403) = 17.31$, $p < .001$] and field [$F(5,403) = 3.20$, $p < .01$] and a two-way interaction of drug by field [$F(10,403) = 6.27$, $p < .05$]. Mean and standard error of the mean for movement, other behaviours and bodyweight are shown in Table 1.

While females in the sham group fed water tended to move more than males in the corresponding group, mean movement for all male groups was higher than that for all female groups (Table 1). In sham groups, administration of LA increased movement. Rats given 5, 50 or 500 nT but not 1 or 10 nT fields moved significantly more than untreated shams (Fig. 1).

Strong interactions occurred between field intensity and NO modulation. While NMA had no effect on shams, co-administration of NMA with field significantly counteracted or retarded all field-induced movement increases in 5, 50 and 500 nT groups. Though LA increased movement in shams, co-administration of any field antagonized this ability. The antagonism was proportional to field intensity. The linear term was shown to be significant ($F(1,150) = 31.61$, $p < .001$).

3.2. Rearing

Three-way ANOVA detected significant main effects for drug [$F(2,403) = 3.20$, $p < .01$] and field [$F(5,403) = 3.20$,

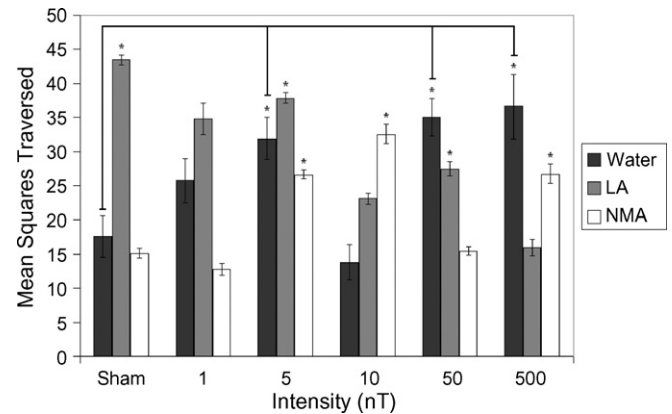


Fig. 1. Mean and S.E.M. for movement by treatment group. For comparison, the experimental field-treated (5, 50 and 500 nT) groups are shown linked to shams. Groups that are significantly different from shams are labeled with an asterisk.

$p < .01$) and a significant drug by field interaction [$F(10,403) = 3.20$, $p < .01$].

Similar to the effects on movement, administration of LA increased rearing while NMA reduced it. Both 10 and 500 nT fields also increased rearing. The rearing totals of 500 nT-treated rats were higher than most groups but were not different than those for 50 nT fields.

Complex intensity-dependent interactions with LA were observed. Groups given LA + 5 nT or LA + 10 nT field showed increased rearing even though neither field had an effect on its own, supporting a weak partial synergy between LA and the field. However, when co-administered with 500 nT EMF, LA counteracted the ability of the field to increase rearing. At all intensities, NMA administration antagonized field-mediated changes in rearing.

Table 1

Mean and standard error of the mean (S.E.M.) for bodyweight and all behaviours in the open field by treatment group

Group	n	Weight	Squares traversed	Rearing	Grooming
Sh	17	36.06 (0.99)	17.59 (3.00)	4.06 (0.71)	0.47 (0.12)
Sh/LA	33	42.03 (0.73)	43.45 (3.21)	7.06 (0.61)	1.06 (0.14)
Sh/NMA	28	26.75 (0.72)	15.14 (3.55)	2.14 (0.32)	0.89 (0.17)
1nT	30	42.13 (0.97)	25.77 (3.25)	3.33 (0.41)	0.77 (0.14)
1nT/LA	23	50.08 (2.31)	34.78 (4.23)	3.87 (0.49)	0.65 (0.16)
1nT/NMA	24	33.13 (0.88)	12.75 (2.63)	1.46 (0.29)	0.88 (0.16)
5nT	33	40.57 (0.80)	31.88 (3.02)	4.09 (0.50)	1.09 (0.13)
5nT/LA	27	46.70 (0.71)	37.81 (4.06)	5.89 (0.78)	0.59 (0.14)
5/NMA	32	30.34 (0.65)	26.59 (5.34)	3.50 (0.39)	0.66 (0.15)
10nT	16	34.44 (0.41)	13.81 (2.56)	2.94 (0.47)	0.75 (0.19)
10nT/LA	29	45.21 (0.85)	23.10 (2.90)	6.24 (0.66)	0.69 (0.15)
10nT/NMA	10	41.70 (1.37)	32.50 (6.48)	7.30 (0.84)	0.90 (0.31)
50nT	27	47.11 (1.35)	35.04 (2.78)	5.56 (0.55)	0.67 (0.13)
50nT/LA	29	46.52 (0.95)	27.41 (2.81)	4.38 (0.45)	0.83 (0.13)
50nT/NMA	27	36.41 (0.61)	15.44 (3.22)	1.70 (0.34)	0.74 (0.13)
500nT	22	56.05 (0.81)	36.64 (4.82)	11.50 (1.54)	1.32 (0.37)
500nT/LA	15	38.73 (1.21)	16.00 (3.55)	4.33 (0.66)	0.73 (0.18)
500nT/NMA	17	44.82 (1.37)	26.65 (4.09)	2.53 (0.56)	0.47 (0.12)

Sh = Sham.

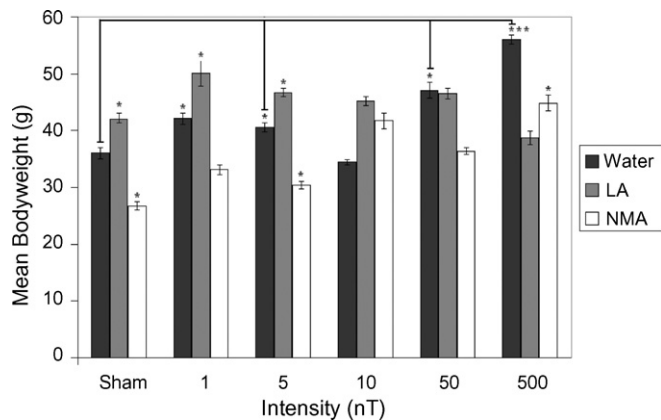


Fig. 2. Mean and S.E.M. for bodyweight by treatment group. For comparison, the experimental field-treated (5, 50 and 500 nT) groups are shown linked to shams. A single asterisk denotes groups significantly different than shams. The triple asterisk denotes that the 500 nT + water group is significantly different from all other groups.

3.3. Grooming

Three-way ANOVA detected a significant interaction of drug by field [$F(10,403) = 8.54, p < .001$] but no main effects. Elevations in grooming occurred with exposure to both 5 and 500 nT fields. These increases were inhibited by administration of either NMA or LA. Both NMA and LA applied singly were found to increase grooming.

3.4. Defecation and urination

Three-way ANOVA yielded no significant findings for defecation or urination.

3.5. Bodyweight

Three-way ANOVA detected significant main effects for sex [$F(1,403) = 54.75, p < .001$], drug [$F(2,403) = 211.82, p < .001$] and field [$F(5,403) = 51.29, p < .001$] and a significant two-way interaction between drug and field [$F(10,403) = 28.37, p < .001$].

Combining all treatment groups, males were heavier than females. NO modulation with either drug had a strong effect on bodyweight. Rats given LA were significantly heavier than those fed water while those fed NMA were lighter (Fig. 2). Field treatment was shown to have a potent effect on bodyweight at all intensities except 10 nT. Exposure to 1, 5, 50 or 500 nT fields increased bodyweight with the increases at 50 and 500 nT being stronger than those at lower intensities.

Administration of LA potentiated the bodyweight increases that occurred with exposure to 1 and 5 nT fields. A reverse trend was noted at higher field intensities as LA again counteracted field-mediated effects. Rats given 500 nT + LA were not significantly different from controls despite the potent ability of the field to increase bodyweight. At all intensities, NMA administration counteracted or cancelled field-mediated increases in bodyweight.

4. Discussion

In the present study, the interaction between perinatal exposure to EMF and NO modulation with either 0.1 g/L NMA or 1 g/L LA was examined. LA administration increased movement, rearing and bodyweight in the open field in 22-day-old pups while NMA administration reduced rearing and bodyweight. Robust EMF-induced increases in movement and bodyweight were noted at 5, 50 and 500 nT and closely resembled the effects of NO modulation with LA. Co-administration of NO inhibitor NMA with EMF counteracted most field-mediated effects at virtually all field intensities. Co-administration of LA with EMF had little effect at low intensities (<5 nT) and strongly counteracted field-mediated effects at higher intensities. Together these results support a strong developmental role of NO and a complex role of NO in EMF effects.

4.1. Effects of the 7 Hz field

While the 7 Hz waveform has been used in several experiments (Cook and Persinger, 2000; Persinger et al., 2000; Kinoshameg and Persinger, 2004), this is the first investigation into any developmental effects from exposure. Here, perinatal exposure to the field significantly impacted motor behaviour in a manner consistent with previous studies which used intensities many times greater (Persinger, 1969; St. Pierre and Persinger, 2003; McKay et al., 2003a,b). The effects seen here were pronounced at the higher intensities as the 50 and 500 nT-exposed rats exhibited dramatic increases in movement and bodyweight. While the effect of the 5 nT EMF is unusual given the extremely weak field intensity, measurable effects in humans have been reported with field intensities nearly a thousand times lower (Sandyk, 1994). Intensity-windowing, as suggested by Blackman (2006) was evident as 5, 50 and 500 nT fields were more effective than 1 or 10 nT fields and tended to influence more variables more strongly.

Even though completely unexpected, the increases in bodyweight that resulted from field exposure were more powerful than those for movement. Marginal bodyweight increases were found at 1 and 5 nT and pronounced increases were found at 50 and 500 nT. While bodyweight changes are a feature of chronic NO modulation (Voelker et al., 1995; Prickaerts et al., 1998; McKay et al., 2003a), they are not a feature of EMF and appear specific to the 7 Hz waveform. The mechanisms underlying these changes are as yet unknown.

Field-mediated rearing increases were noted at 5 and 500 nT while grooming increases were found only at 10 and 500 nT. These anomalies, when accompanied with abnormal movement, may indicate that a maladaptive phenotype is being observed. Rearing is thought to be a form of exploratory behaviour (Archer, 1973) and abnormal rearing may be an indicator of impairment. Modulation of rearing behaviour occurs with acute NO modulation in the component models of attentional disorders (Aspide et al., 1998, 2000; Grammatikopoulos et al., 2002) which could suggest attention has also been

affected by the treatments used here. Alternatively, since EMF (Verkasalo et al., 1997) and NO modulation with LA (Ergun and Ergun, 2007) may be pro-depressant, it is possible that psychogenic factors were involved in open field performance. Another possibility is that the increased grooming seen in LA- and field-treated animals is exceptionally high and may be an indicator of elevated stress or frustration (McFarland, 1966). The role of NO in the stress response appears key but is still being determined (Echeverry et al., 2004).

4.2. Effects of pharmacological NO modulation

NO inhibition by NMA reduced rearing and bodyweight significantly while NO donation with LA, in addition to increasing movement, had the opposite effect. The effects of maternal administration of LA suggest that high levels of the drug may be aversive to the developing fetus or infant in ways previously overlooked. Effects of LA administration on bodyweight similar to those here have been reported in many young animals (Fligger et al., 1997; Wu et al., 2004; Deng et al., 2005) but results in adult rats (Tsubuku et al., 2004) and humans (Evans et al., 2004) are largely negative. Both age and species play a prominent role in the effects of LA on bodyweight. Unfortunately, comparative studies in young humans are lacking, as is longitudinal data, so it is difficult to tell the time course of these effects or if they can be elicited in young humans.

In examining the role of NO in EMF response, most studies utilize injections of an NO inhibitor into the subject (Dixon and Persinger, 2001; Jeong et al., 2006). In the preponderance of these experiments, acute injection of an NO inhibitor reverses or blocks most field-mediated effects. Here, as found by McKay and others (McKay et al., 2003a), a reversal of field-mediated effects was possible even though the subject received no direct injection and acquired the drug through a maternal route. The NO inhibitor NMA reversed nearly all field-mediated effects seen in offspring, including the dramatic increases in movement, rearing and bodyweight seen in 500 nT-exposed animals. Comparatively weak effects were evident in the sham field exposed rats. The methods here suggest that chronic modulation of NO through non-invasive means is not significantly different from acute NO modulation by injection.

Evaluation of potential interactions between NO donors and EMF is not commonly a feature of EMF studies, but some evidence supports that synergies may exist (Kavaliers et al., 1998) in that NO donation amplifies the effects of EMF treatment. Here, only weak interactions between EMF and LA were noted at low intensities (<5 nT). Ironically, the two treatments were antagonistic to each other as LA administration counteracted the effects of higher intensity fields. In the case of movement, this negative interaction even followed a linear trend. Co-administration of LA antagonized the increases in movement and rearing seen with exposure to 50 or 500 nT fields and the bodyweight increases seen with exposure to 500 nT fields. While other pharmacological substances have potent synergies with EMF (Whissell and Persinger, *accepted*), augmentation of EMF effects with NO donation has been difficult to demonstrate experimentally.

5. Conclusion

Within the past 20 years, many new, EMF-based treatments for disease and disorder have evolved (Liboff, 2004) which are gaining in popularity. Additionally, many medications and popular supplements that directly influence NO activity such as LA are widely used in the treatment and nutritional programs. Critical to these endeavours is an understanding of the effects of EMF, NO, and any interactions between the two, particularly within the perinatal context in which a flexible nervous system and physiology are under development.

The interactions seen here show conspicuous effects of perinatal EMF exposure that are highly similar to those of NO modulation. As EMF and NO-based treatments are being applied to increasingly large clinical populations, further experiments will be needed to characterize any potential side effects or contra-indications that may emerge with widespread use.

Acknowledgements

The authors would like to thank Chris Blomme, Professor Linda St. Pierre and Dr. Cynthia Whissell for technical assistance. Funding for this experiment was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Foundation for the Study of Infant Deaths (Dr. S. Segal Research Grant).

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